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REVIEW ARTICLE

Spectral Methods for the Characterization of Polymorphs and Solvates

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Introduction

Although new routes of administration for pharmaceutically active therapeutic agents continue to be developed, most compounds are still administered as a solid dosage form.[1] Any defining characteristic that might affect the stability or availability of the drug substance in a solid dosage form should be monitored and controlled,[2] so, the physical characterization of solids has become an extremely important area in pharmaceutics.[3,4] Important investigations into solid-state phenomena can center on questions of chemical reactivity, where attention is focused on the solid-state reactions that occur in bulk drugs or in their dosage forms.[6] An equally important area of solid-state pharmaceutics is the study of the crystallographic properties of a given compound. Indeed, crystallography studies are sometimes carried out solely with the intention of determining possible variations in the structural aspects of solid forms of drugs.[6]

Byrn *et al.*[7] have provided a series of useful definitions that concisely give the characteristics of the various solid forms that may be found for a given drug substance.[7] Compounds may be **polymorphs** (forms with the same chemical composition but different crystal structures), **solvates** (forms containing solvent molecules within the crystal structure), **desolvated solvates** (forms from which the solvent is removed from a specific solvate and that still retain the original crystal structure), or **amorphous** (solid forms that have no long-range molecular order). A full evaluation of possible variations in crystallography that might be encountered is now essential for the development for a new drug compound because the Food and Drug Administration (FDA) requires that analytical procedures be used to detect polymorphic, hydrated, or amorphous forms of the drug substance. A series of flow charts and decision trees have been presented that are to be used by investigators seeking to characterize the crystallography of compounds under development for registration with regulatory authorities.[8]

The pharmaceutical consequences of polymorphism and solvate formation have been known for some time,[9] because it has been recognized that differential crystal structures of a given chemical entity might exhibit differing solubilities, stabilities, or bioavailabilities. The occurrence of polymorphism is quite common for organic molecules, and a great number of polymorphic drug compounds have been noted and cataloged.[10-12] A complement of physical characterization methods have been developed for the study of polymorphs and solvates,[3,4,13] with many workers choosing to use the classical methods of crystallography, microscopy, thermal analysis, and solubility studies. However, it must be emphasized that the defining criterion for the existence of polymorphic types is a nonequivalence of crystal structures. For pharmaceutical agents, this criterion requires that nonequivalent X-ray powder patterns are observed for the various forms. All other observations must be considered as supporting and ancillary information and cannot alone be taken as definitive proof of the existence of polymorphism.

Once the existence of polymorphism (or solvate formation) is definitely established by X-ray diffraction, spectral methods of analysis can be of great value. Vibrational spectroscopy [infrared (IR) absorption or Raman scattering] contains information about the motions of functional groups in the solid and is often site-specific in nature. If the molecular vibrations are affected by the structural differences that characterize the polymorphism, then studies of the vibrations will be useful for an evaluation of the origin of the effect.[14] Similarly, nuclear magnetic resonance (NMR) can be used to probe the environments of atoms in the solid state, and structurally nonequivalent nuclei would resonate at observably different frequencies.[15] Either approach can be used to study the structural variations that exist in solvate species.[16]

In this article, the use of spectral techniques (primarily vibrational spectroscopy and NMR) performed on the solid-state materials for the study of polymorphs and solvates will be reviewed. No attempt will be made to summarize every recorded use of these spectroscopic techniques for such characterization work, but selected examples will be used to illustrate what type of information can be extracted from the implementation of appropriate methodology. Very limited use has been made of electronic spectroscopy, but some applications have been reported and these will also be discussed.

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Vibrational Spectroscopy

The energies associated with the vibrational modes of a chemical compound lie within the range 400–4000 cm^{-1}. These modes can be observed directly through their absorbance in the IR region of the spectrum, with Fourier-transform IR (FTIR) spectroscopy now being the method of choice. Most workers are familiar with the use of mid-IR spectra for identity purposes, where the pattern of absorption bands is taken to be diagnostic for a given compound. In addition, extensive compilations of group vibrational frequencies exist that allow the ready assignment of observed bands.[17]

The acquisition of high-quality IR spectra on solid materials is most amenable with the FTIR method, because that approach minimizes transmission and beam attenuation problems.[18] Essentially all FTIR spectrometers use a Michelson interferometer. Radiation entering the interferometer is split into two beams with a beam splitter. One beam follows a path of fixed distance before being reflected back into the beam splitter, and the other beam travels a variable distance before being recombined with the first beam. The recombination of these two beams yields an interference pattern, where the time-dependent constructive and destructive interferences have the effect of forming a cosine signal. Each component wavelength of the source will yield a unique cosine wave, with a maximum at the zero pathlength difference (ZPD), that decays with increasing distance from the ZPD. The detector is placed so that radiation in the central image of the interference pattern will be incident upon it, and therefore intensity variations in the recombined beam are manifest as phase differences. The observed signal at the detector is a summation of all the cosine waves, with a maximum at the ZPD, that decays rapidly with increasing distance from the ZPD. If the component cosine waves can be resolved, then the contribution from individual wavelengths can be observed. The frequency domain spectrum is obtained from the interferogram by performing the Fourier transformation mathematical operation.

Alternatively, the vibrational modes of a compound may be measured by Raman spectroscopy, where one measures the inelastic scattering of radiation by a nonabsorbing medium.[19] When a beam of light is passed through a medium, approximately one in every million incident photons is scattered, with a loss or gain of energy. The inelastic-scattered radiation can occur at lower (Stokes lines) and higher (anti-Stokes lines) frequencies relative to that of the incident (or elastically scattered) light, depending on the vibrational transition frequencies of both the sample or medium. The actual intensities of the Stokes and anti-Stokes lines are determined by the Boltzmann factor that characterizes the vibrational population. For high-frequency vibrations, the Stokes lines are relatively intense compared with the anti-Stokes lines, so conventional Raman spectroscopy makes exclusive use of the Stokes component. The Raman effect originates from the interaction of the oscillating-induced polarization or dipole moment of the medium with the electric field vector of the incident radiation. Raman spectra are measured by passing a laser beam through the sample and observing the scattered light either perpendicular to the incident beam or through back-scatter detection. The scattered light is analyzed at high-resolution by a monochromator and ultimately, detected by a suitable device. The key to obtaining good spectra is to use a notch filter that will eliminate the exciting line, which is required to obtain acceptable signal-to-noise ratios.

In the best designed studies of polymorphic or solvate systems, the purpose of the spectral investigation should be to gather information about the underlying structural aspects that give rise to the observed crystallographic differences. Once suitable spectral features are identified from this work, they can be used to develop methods for the quantitative analysis of one polymorph (or solvate) in the presence of the other. Although most use has been made of spectral features observed in the mid-IR region,[20] quantitative methods have been advanced that make use of the overtone bands that occur at energies within the near-IR region.[21,22]

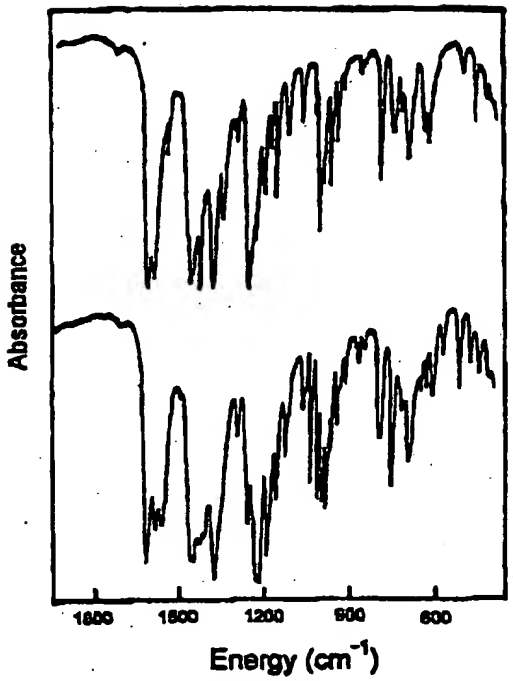

Figure 1—Infrared absorption spectra obtained within the fingerprint region for ranitidine hydrochlorides; Form I (upper trace) and Form II (lower trace). The data were adapted from ref 24.

Perhaps the best-known occurrence of polymorphism concerns the two forms of ranitidine hydrochloride, for which a specific polymorph has been patented[23] and for which the crystallography serves to protect the innovator from generic incursions. As shown in Figure 1, the IR spectra of the two forms of ranitidine hydrochloride contain substantial differences within the fingerprint region, with the band at 1045 cm^{-1} being most useful for diagnostic purposes.[24] At the same time, a number of the spectral features are seen to be equivalent in the two structures. With the proper assignment of equivalent and nonequivalent bands to molecular vibrations, it is possible to use vibrational spectroscopy to deduce the molecular origins of the observed polymorphism.

The IR spectra of the polymorphs of acetohexamide and selected derivatives have been used to study the tautomerism of the drug compound by Takla and Dakas.[25] Was deduced that Form A existed in the enol form, which was stabilized by the intramolecular bonding between the O—H and S—O groups that produces a six-membered ring. Form B was characterized by the existence of the keto form, with the urea carbonyl group bonded intermolecularly to a sulfonamide N—H functionality. This behavior may be contrasted with that noted for spironolactone, for which no evidence was found for the existence of enolic tautomers in any of the four polymorphs.[26]

When a carbonyl group is present in a compound, the energy associated with its stretching mode is often reflective of any structural or conformational polymorphism. In fosinopril sodium, the energies of two equivalent carbonyls were similar in the two polymorphic forms, but the energy of the third carbonyl (present in nonequivalent side chains) was found to differ in the two forms.[27] Differing modes of hydrogen bonding

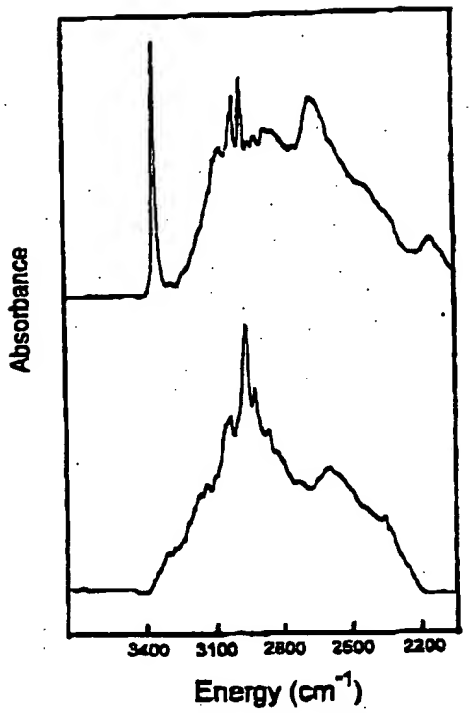

Figure 2—Infrared absorption spectra obtained within the oxygen-hydrogen stretching region for ampicillin trihydrate (upper trace) and anhydrate (lower trace). The data were adapted from ref 39.

in different polymorphs yielded different frequencies for the carbonyl stretch in tegafur,[28] and structural variations were postulated to account for the trends in carbonyl frequencies of moricizine hydrochloride.[29]

Solvent molecules can often be incorporated in a crystal lattice, resulting in the formation of solvate structures that differ from those of the analogous anhydrate structure or structures.[16] For compounds of pharmaceutical interest the solvent of interest is normally water, because the occlusion of other residual solvents is ordinarily a cause for concern to regulatory authorities. Etoposide is a perfect example of how water can complicate the crystallography of a compound.[30] Form I of this compound is a monohydrate, but can be dehydrated to Form Ia by heating to 85–115 °C. Form Ia melts at 198 °C, but will crystallize to Form IIa if heated to 206 °C. Form IIa will convert to another hydrate (Form II) if it is exposed to ambient humidity at room temperature. Forms I and II exhibit differing solubilities, so control of the phase composition is important to ensure consistent dissolution. In this particular system, the IR spectra of the two forms are nearly identical, requiring the use of X-ray powder diffraction for evaluation purposes.[30]

Solid-state IR spectroscopic studies have figured prominently in the characterization of the polymorphism associated with carbamazepine,[31] triamterene,[32] estramustine,[33] felodipine,[34] mexiletine hydrochloride,[35] ranitidine hydrochloride,[36] diflunisal,[37] and zanoterone.[38]

The crystalline water of a hydrate system can often be detected in the oxygen-hydrogen stretching region of the IR spectrum as a sharp peak located at a higher energy than any of the other hydrogen-stretching modes. This phenomenon has been illustrated in Figure 2 for ampicillin, where the unique absorption assignable to lattice water in the trihydrate phase is totally resolved from the other high-frequency absorption bands.[39] As illustrated by the example of oxyphenbutazone, the distinction between hydrate and anhydrate phases is not always so distinct in the IR spectrum,[40] but the presence of crystalline water can usually be deduced from the spectra.

In other systems, the presence of water molecules in the crystal lattice is sufficient to affect the vibrational bands that are observed in the fingerprint region. The monohydrate, trihydrate, and pentahydrate forms of urapidil each exhibit differing absorptions in the carbonyl region, indicating that the various hydrogen-bonding arrangements of the respective solids perturb the carbonyl stretching mode in different ways.[41] A similar variability in IR spectra was noted in a study of the eight anhydrate and two hydrate phases of mefloquine hydrochloride.[42]

Although solvates other than hydrates are not ordinarily of importance as drug substances, they represent situations where IR spectroscopy can be fruitfully used to study the crystallography. For example, the IR spectra of the pentanol and toluene solvates of glibenclamide were readily distinguishable from spectra obtained on either of the two crystalline anhydrate phases.[43] This situation is particularly evident in the IR spectrum of 9,10-anthraquinone-2-carboxylic acid, where the hydroxyl stretching mode of the crystalline methanolic solvate is particularly well-resolved from the remainder of the proton stretching vibrations.[44]

Infrared spectroscopy has been used to obtain additional information on the methanol solvate of urapidil,[45] the *N,N*-dimethylformamide and dioxane solvates of furosemide,[46] the dioxane solvate of phenobarbital,[47] and the dimethyl sulfoxide and 1-methyl-2-pyrrolidone solvates of 3-amino-1-(*m*-trifluoromethylphenyl)-6-methyl-1*H*-pyridazin-4-one.[48]

Although both IR absorption and Raman scattering yield information on the energies of the same vibrational bands, the different selection rules governing the band intensities for each type of spectroscopy can yield useful information. For the low-symmetry situations presented by the structures of molecules of pharmaceutical interest, every vibrational band will be active in both IR absorption and Raman scattering spectroscopies. The relative intensities of analogous bands will differ, however, when observed by either IR absorption or Raman spectroscopy. In general, symmetric vibrations and nonpolar groups yield the most intense Raman scattering bands, whereas antisymmetric vibrations and polar groups yield the most intense IR absorption bands. This type of behavior is illustrated in Figure 3, where both types of vibrational spectroscopy were used to study the polymorphic modifications of nimodipine.[49] It is evident from the intensity relations that although each technique yields a summary of the vibrational transitions, substantial differences in band intensity are readily discernible.

Nuclear Magnetic Resonance Spectrometry

The ultimate characterization of a pharmaceutical material will get down to the level of individual chemical environments of each atom in the compound, and this information is best obtained through the use of NMR spectroscopy. With recent advances in instrumentation and computer pulse sequences, these studies can now be routinely carried out in the solid state.[50] Although any nucleus that can be studied in the solution phase can also be studied in the solid state, most of the work has focused on ^{13}C studies. As in the case of vibrational spectroscopy, extensive compilations of ^{13}C resonances for various functional groups are available in the literature,[51] and these can prove useful during the initial assignments of resonances to functional groups.

Proton NMR ^{1}H NMR remains an extremely difficult measurement in the solid state, and the data obtained from such work can only be obtained at medium resolution. The

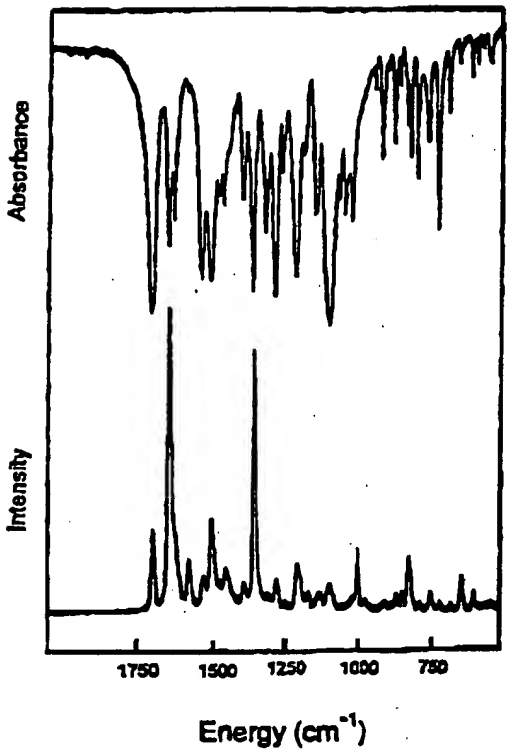

Figure 3—Infrared absorption (upper trace) and Raman (lower trace) spectra obtained for nimodipine, Form II. The data were adapted from ref 49.

field acting at the nucleus is affected by the magnetic dipoles of neighboring nuclei, and the local fields thus generated are sensitive to both the internuclear distances and their orientation relative to the external field. Protons are abundantly present in organic compounds, so the removal of proton—proton dipolar interactions is necessary to obtain high-resolution ^1H NMR spectra in solids. Although the removal of proton—proton dipolar interactions is possible, the resulting ^1H NMR spectra are still inferior to those obtained in the solution phase. The primary reason for this inferiority is that ^1H NMR has one of the smallest isotropic chemical shift ranges (12 ppm) but peak broadening effects that can span several ppm in magnitude. Other nuclei yield far better data, with ^{13}C and ^{31}P solid-state NMR studies being very useful to the physical characterization of all pharmaceutical solids.

The local magnetic field (B_{loc}) at a ^{13}C nucleus in an organic solid is given by:

$$B_{loc} = \pm\{h\,\gamma_H/4\,\pi\}\{(3\cos^2\theta - 1)/r^3\} \qquad (1)$$

where γ_H is the magnetogyric ratio of the proton, r is the internuclear C—H distance to the bonded proton, and θ is the angle between the C—H bond and the external applied field (B_o). The ± sign results from the fact that the local field may add to or subtract from the applied field depending on whether the neighboring proton dipole is aligned with or against the direction of B_o. In a microcrystalline organic solid, there is a summation over many values of θ and r, resulting in a proton dipolar broadening of many kilohertz. A rapid reorientation of the C—H internuclear vectors (such as those associated with the random molecular motions that take place in the liquid phase) would result in reduction of the dipolar broadening. In solids, such rapid isotropic tumbling is not possible; but, because the term $(3\cos^2\theta - 1)$ equals zero if θ equals $\cos^{-1} 3^{-1/2}$ (≈54°44′), spinning the sample at the so-called "magic angle" of 54°44′ with respect to direction of the applied magnetic field results in an averaging of the chemical shift anisotropy. In a solid sample, the anisotropy reflects the chemical shift dependence of chemically identical nuclei on their spatial arrangement with respect to the applied field. It is this anisotropy that is primarily responsible for the spectral broadening associated with ^{13}C samples, so spinning at the magic angle makes it possible to obtain high-resolution ^{13}C NMR spectra of solid materials.

An additional method for the removal of ^{13}C—^1H dipolar broadening is to use a high-power proton decoupling field, often referred to as dipolar decoupling. One irradiates the sample with high power at an appropriate frequency, that results in the complete collapse of all ^{13}C—^1H couplings. With proton dipolar coupling alone, the resonances in a typical solid-state ^{13}C NMR spectrum will remain very broad (on the order of 10—200 ppm). This broadening arises from the fact that the chemical shift of a particular carbon is directional, depending on the orientation of the molecule with respect to the magnetic field.

Even though high-resolution spectra can be obtained on solids by the MAS technique, the data acquisition time is lengthy due to the low sensitivity of the nuclei and the long relaxation times exhibited by the nuclei. This problem is circumvented through the use of cross polarization (CP), where spin polarization is transferred from the high-abundance, high-frequency nucleus (^1H) to the rare, low-frequency nucleus (^{13}C). This process results in up to a fourfold enhancement of the normal ^{13}C magnetization and permits a shortening of the waiting periods between pulses. The CP experiment also allows the measurement of several relaxation parameters that can be used to study the dynamic properties of the solid under investigation.

It is often observed that the NMR spectra of compound polymorphs or solvates contain nonequivalent resonance peaks for analogous nuclei. This effect arises because the intimate details of the molecular environments associated with differing crystal structures can yield a nonequivalent relationship with respect to the applied magnetic field of the NMR experiment, which in turn causes the analogous nuclei to resonate at different energies. As has been already noted for the IR spectra of polymorphs or solvates, it is not uncommon for certain resonance peaks to be observed at identical chemical shifts, whereas other resonances are significantly shifted.[52] It is not difficult to assign organic functional groups to observed resonances, so solid-state NMR spectra can be used to deduce the nature of polymorphic variations. This technique is especially valuable when the crystal polymorphism is conformational in origin.[53] Such information is extremely valuable at the early stages in drug development when solved single crystal structures for each polymorph or pseudopolymorph may not be available.

During the development of fosinopril sodium, a crystal structure was solved for the most stable phase, but no such structure could be obtained for its metastable phase.[27] The compound contains three carbonyl groups and, as shown in Figure 4, the solid-state ^{13}C NMR spectra of two of these are essentially equivalent. The third carbonyl, located on the acetal side chain, resonated at different chemical shifts in the two structures. When combined with the observations obtained using vibrational spectroscopy, these results permitted the deduction that the solid-state polymorphism was associated with different conformations of this side chain.[27] The NMR data also suggested that additional conformational differences between the two polymorphs were associated with *cis-trans* isomerization along the peptide bond, that in turn results in the presence of nonequivalent molecules existing in the unit cell. In the absence of solved crystal structures

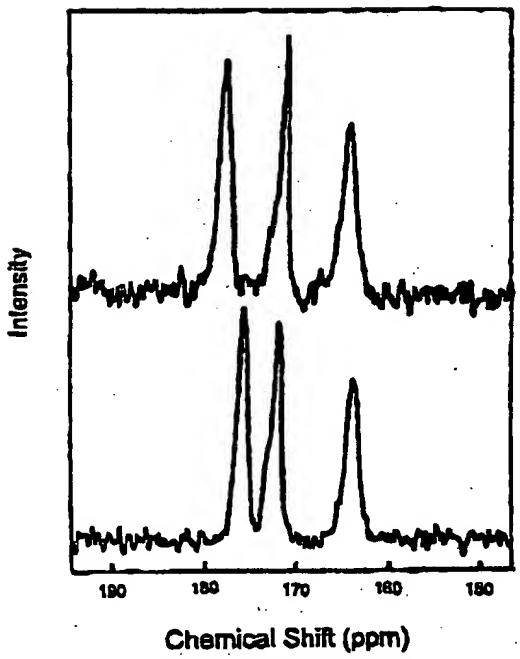

Figure 4—Solid-state ^{13}C NMR spectra obtained within the carbonyl region of fosinopril sodium: Form A (upper trace) and Form B (lower trace). The data were adapted from ref 27.

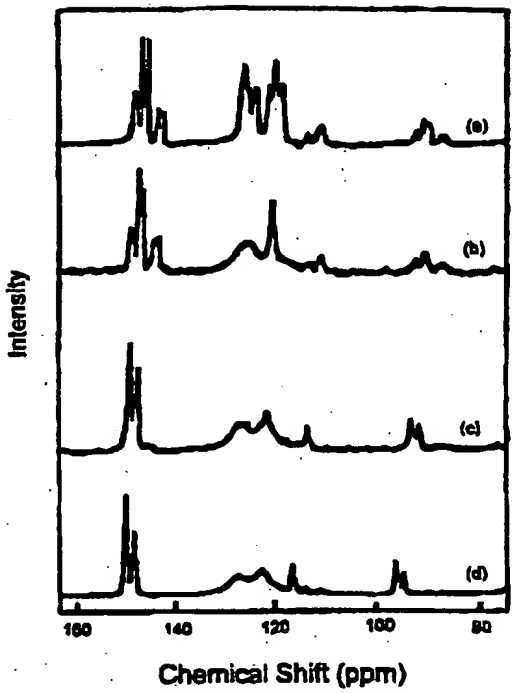

Figure 5—Solid-state ^{13}C NMR spectra obtained at various temperatures for 2,2-bis(*p*-hydroxyphenyl)propane, Form II, at nominal temperatures of (a) ambient, (b) 45 °C, (c) 55 °C, and (d) 75 °C. The data were adapted from ref 60.

for the two polymorphs, this information would not have been otherwise obtainable.

The solid-state ^{13}C NMR spectra of the two polymorphs of furosemide revealed the existence of altered chemical shifts and peak splitting patterns indicative of differences in molecular conformations.[54] Studies[54] of $T_{1\rho}$ relaxation times were used to show the presence of more molecular mobility and disorder in Form II, whereas the structure of Form I was judged to be more rigid and uniformly ordered. During a solid-state spectroscopic study of the polymorphs of losartan, it was deduced that the spectral characteristics of Form I implied the presence of multiple orientations for the *n*-butyl side chain and the imidazole ring.[55] It was also concluded that Form II was characterized by a large molecular motion of the *n*-butyl side chain.

Not all polymorphism originates from conformational requirements, and many polymorphic situations exist as a result of different modes of molecular packing in the solid-state structures. For example, the two polymorphs of enalapril maleate exhibit very similar molecular conformations (as evidenced by the similarity in spectral characteristics), with the observed differences in crystal structure therefore being attributed to different modes of crystal packing.[56] Sufficient differences in the solid-state ^{13}C NMR spectra of the four polymorphs of sulfathiazole were observed that would enable the use of this technique as an analytical tool, but these differences could not be ascribed to differences in molecular conformations among the polymorphs.[57]

The power of NMR spectroscopy can be significantly enhanced through use of the numerous available techniques for data acquisition. For example, the analysis of the solid-state ^{13}C NMR spectra of (1*R*,3*S*)-3-*p*-thioanisoyl)-1,2,2-trimethylcyclopentanecarboxylic acid was facilitated by the *J*-modulated spin-echo technique, that was used to deduce the number of protons bound to each carbon atom.[58] Differences in the dipolar dephasing behavior between the two polymorphs of (±)-*trans*-3,4-dichloro-*N*-methyl-*N*-[1,2,3,4-tetrahydro-5-methoxy-2-(pyrrolidin-1-yl)]naphth-1-yl-benzeneacetamide were noted, and ascribed to motional modulation of the carbon–hydrogen dipolar interaction.[59] This added degree of molecular motion was used to deduce a loosely packed crystal structure for Form II of this compound.

The acquisition of solid-state ^{13}C NMR spectra at various temperatures can be a powerful approach to the study of molecular motion in solids, and for the study of phase conversion. Such methodology was used to study the two polymorphs of 2,2-bis(*p*-hydroxyphenyl)propane, where markedly different molecular mobilities were found to exist in the two crystal structures.[60] Below the glass transition temperature, the important active mode correlates with the flipping of the phenylene rings, and this effect was evident in the NMR spectra obtained at different temperatures. Some of the resonance bands associated with Form II coalesced upon sample heating from −10 °C to ambient probe temperature. As illustrated in Figure 5, additional changes in the spectra took place with continued heating, and Form II convert to Form I at a nominal temperature of 65 °C.

Solid-state NMR spectroscopy can also be used to study the molecular environments of nuclei as these vary in the differing structures associated with solvates and hydrates. For example, the solid-state ^{13}C NMR spectra obtained within the carbonyl region for the anhydrate and monohydrate phases of oxyphenbutazone differed between the two structures.[61] The crystal structures of both forms were available, so the observed spectral differences were ascribed to the effects of hydrogen bonding between one water molecule and the carbonyl group, as well as hydrogen bonding between another water molecule and the phenolic group. The lack of these hydration waters in the anhydrate phase changes the overall structure, perturbing the molecular environments of the affected nuclei.

One anhydrate (Form α) and two polymorphic monohydrate phases (Forms β and δ) of testosterone have been crystallographically characterized, and the solid-state ^{13}C NMR spectra have been obtained for each.[62] The analysis of the spectra

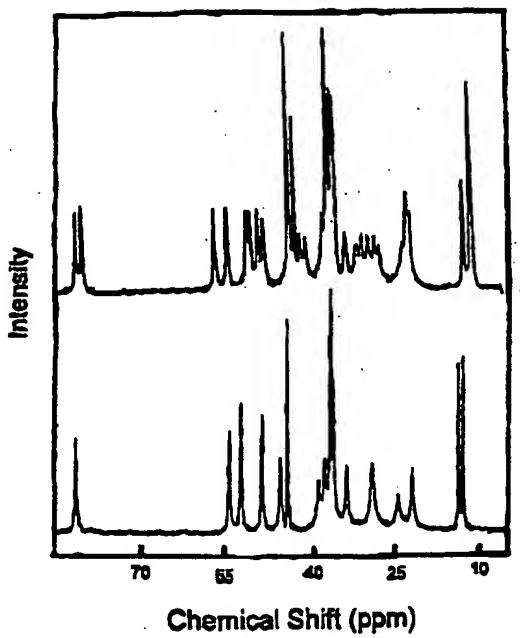

Figure 6—Solid-state ^{13}C NMR spectra obtained within the aliphatic carbon region of the anhydrate (upper trace) and monohydrate (lower trace) forms of androstanolone. The data were adapted from ref 63.

was complicated by the observation that many carbons of a given form resonated as doublets, reflecting the situation that more than one molecular type existed within the unit cells. In a subsequent study, the solid-state ^{13}C NMR spectra were obtained on the anhydrate and monohydrate phases of androstanolone (a known metabolite of testosterone.[63] The spectra obtained within the aliphatic carbon region for the two forms are shown in Figure 6, where many doublets (arising from incongruences in the unit cell) were found in the anhydrate spectrum. In the monohydrate phase, no such doubling was observed because the two molecules present in the unit cell are related by symmetry and consequently magnetically equivalent.

Dirithromycin presents an array of anhydrous and solvated structures and is known to crystallize in two anhydrate forms and at least nine stoichiometric solvate forms. In a study of several of these forms, it was found that the differences in intermolecular hydrogen bonding networks became manifest in the solid-state ^{13}C NMR spectra.[64] Distinct chemical shifts were recorded for hydrogen-bonded and nonhydrogen-bonded lactone functionalities, and the NMR spectra were useful in distinguishing among different solvates of similar crystallographic structure.

Electronic Spectroscopy

All molecules are characterized by the existence of a single wave function whose energy is less than that of all others, which is termed the ground state of that molecule. The same molecule will be characterized by the existence of numerous excited states whose energies are higher than that of the ground state and that consist of wave functions with dissimilar symmetry properties. In the process of molecular absorbance, energy will be absorbed by the molecule as a transition is made from the ground state to one of the excited states. Alternatively, energy will be spontaneously emitted by the molecule if the transition originates in the excited state and terminates in the ground state (molecular fluorescence). Because of the differences in energy between the electronic states of a molecule, the absorption or fluorescence processes are observed in either the visible (vis, 400–800 nm) or ultraviolet (UV, 180–400 nm) regions of the electromagnetic spectrum. In either spectroscopic experiment, an allowed transition is one that is characterized by a change in the orbital angular momentum of the system, with no accompanying change in the spin angular momentum.

The occurrence of absorption bands in the spectrum of an organic molecule is often associated with the presence of specific groups in the molecule (termed chromophores). The energy required for the transition is determined primarily by the details of the molecular orbitals describing the chromophore and is often influenced only to a secondary degree by other atoms or groups for that the electronic binding is very different. For example, aldehydes and ketones containing an unconjugated carbonyl group exhibit a characteristic absorption band with a maximum around 280–290 nm. If a molecule contains more than one chromophore and the chromophores are separated from each other by more than one sigma bond, then the chromophores will undergo independent transitions and the observed spectrum will be a superimposition of these.

Most pharmaceutical solids are too opaque to permit the use of transmission UV/vis electronic spectroscopy and require that investigations be performed with diffuse reflection techniques.[65,66] The simplest reflectance experiment is performed by merely exposing the solid to white light and using the human eye as a detector to sense the color of the solid. This method is more versatile than one might think, because the vision of most individuals is sensitive to literally millions of colors, hues, and shadings. In the instrumental method, the sample is irradiated with monochromatic energy of known and continuously varying wavelength, and one measures the amount of radiant energy reflected from the sample surface. The diffuse reflectance component is defined as reflected energy that has been partially absorbed and partially scattered by a surface with no defined angle of reflectance. The reflectance is collected with an integrating sphere and quantitated by a photomultiplier tube or its equivalent.

The perceived color of a solid is determined by its pattern of light absorption. If a solid is bathed in white light and does not possess any absorption transitions in the visible region of the spectrum, then it will reflect all incident light and appear white to the observer. In general, the color of an absorptive solid will consist of how the human eye perceives the combination of all reflected wavelengths. For example, a solid will appear yellow to an observer if it contains a weak absorption in the blue region of the spectrum, but will appear red if the blue absorption is stronger.

That the color of a given compound can be affected by its polymorphic type has been known for a very long time, although the better-known examples are associated with pure elements.[67] The example of carbon comes immediately to mind, where black (graphite) and colorless (diamond) polymorphs are known. There are two polymorphs known for white phosphorous, both of that consist of discrete tetrahedral P_4 molecules. White phosphorous can be transformed into a black polymeric form by heating or through the application of pressure. A red allotrope is also known, which is a largely amorphous form consisting of disordered P_4 molecules and various polymers of these.

Acridine derivatives can be obtained in a number of polymorphic variations, depending on the details of the crystallization procedure. 2-Hydroxy-5-phenylacridine can be obtained as either a yellow or a red solid, and the yellow polymorph can be converted into the red form by gentle grinding.[68] The two polymorphs differ in a tautomeric transfer of protons, with the structural differences consisting of differing patterns of hydrogen-bonding. Yellow and red

polymorphs of 3-hydroxy-9-phenylacridine have been obtained, where the yellow form consisted of the compound in its lactim state, and where the red form consisted of a mixture of the lactim and lactam forms.[69]

The charge-transfer complex formed by picric acid and 2-iodoaniline can be obtained in two polymorphs, depending on the crystallization process used.[70] Monoclinic Form I contains four cations (2-iodoanilinium) and four anions (picrate) per unit cell and its crystals are yellow in color. The triclinic Form II contains two iodoanilinium cations and two picrate anions per unit cell and exists as a green solid. In Form I, the anion-cation pairs are stacked to form segregated columns in that the same ion types are stacked with each other. In Form II, the anions and cations are alternately stacked to form continuous columns. The structural arrangement of Form I does not allow for the generation of a charge-transfer absorption band, so the solid maintains the yellow color of picric acid. On the other hand, the alternate stacking present in Form II facilitates the overlap of suitable molecular orbitals to yield a new charge-transfer absorption band, which is equally as absorptive as the locally excited band of the picrate anion.

Byrn and co-workers[71] reported a case of color polymorphism associated with the various forms of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile that originated as a result of conformational polymorphism.[71] Crystallization of the compound from ethanol yields a mixture of yellow and red polymorphs, but crystallization from methanol yields an orange polymorph. The crystal structures of these three forms were obtained and used to show that the molecule adopted different molecular conformations in the different structures. The different conformations permit a varying degree of electronic interaction to exist between the planes of the phenyl and the thiophene rings, and the consequent change in molecular orbital energies sufficiently perturbs the electronic transitions so that the apparent color of the solids is affected.

The three crystalline hydrates and liquid crystalline phase of (*S*)-4-[[[1-(4-fluorophenyl)-3-(1-methylethyl)-1*H*-indol-2-yl]-ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, disodium salt, exhibit varying fluorescence properties in their respective solid states.[72] The monohydrate Form I was characterized by an excitation maximum at 345 nm and a fluorescence maximum at 371 nm. Form II (a dihydrate) exhibited essentially equivalent spectral characteristics as did Form I, except for a significant reduction in fluorescence intensity. The excitation maximum found for Form III (effectively a hexahydrate) shifted to 400 nm and the fluorescence shifted to 485 nm. This latter behavior is consistent with the formation of indole excimers in this phase, resulting from a stacking of the ring systems within the solid. The shift in fluorescence maximum that accompanies the conversion of Form I to Form III is illustrated in Figure 7. Finally, upon binding of nine hydration waters, the structure relaxed into a liquid crystalline form that exhibited spectral characteristics similar to those of Form III, but of greatly reduced intensity.

Conclusions

It is now abundantly clear that spectroscopic methods of analysis can be valuable adjuncts for the characterization of polymorphic and solvate systems. However, it must be remembered that the primary determinant of the existence of multiple crystal structures will always be crystallography, and that spectroscopic and other techniques must be considered as ancillary support to x-ray diffraction studies. However, once the genuine existence of polymorphism or solvate formation is established by a crystallographic method, vibrational spectroscopy and NMR spectrometry can be used to deduce valuable information as to the origins of the structural variations.

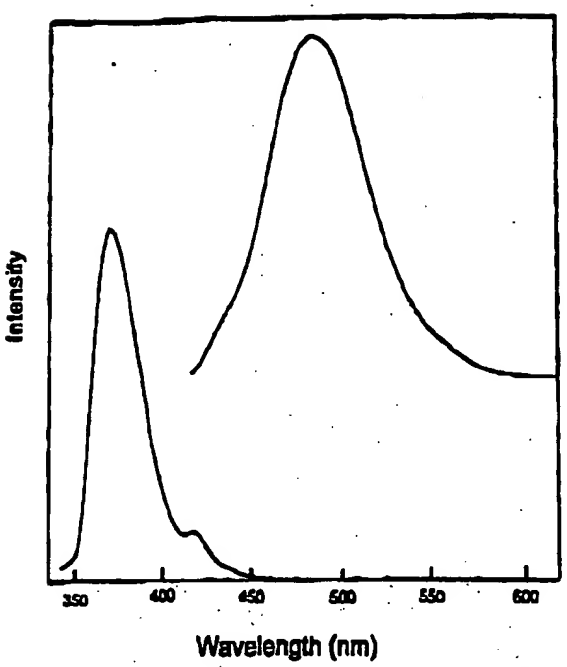

Figure 7—Solid-state fluorescence spectra obtained from the Form III (upper trace) and Form I (lower trace) hydrates of SQ-33600. The data were adapted from ref 68.

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